C5 cond. 201. A stably transformed of a tobacco plant of claim 200 which is a progeny of the tobacco plant.--

Remarks

Interview

Applicant's respectfully request a personal interview with the Examiner when he has reviewed this application.

Extensions of Time

Applicant's respectfully request a three-month extension of time. The necessary fee of $445.00 is enclosed herewith as stated under 37 CFR §1.17(a)(3).

Election

Applicant's election with traverse of Group II of paper No. 9 is acknowledged. The Applicant notes that the Requirement for Restriction is still deemed proper by the Examiner, and is therefore made FINAL.

Regarding the Examiner's remarks, the number of 188 claims (now 201), has been necessary to properly cover the various aspects of the invention.

Regarding claims 185 and 186. The dependency of claims 181, 182, 184, 185, 186 and 188 have been corrected. The undersigned apologizes for the this oversight.

Information Disclosure Statement

The undersigned also apologizes for not having provided an Information Disclosure Statement. Applicant is filing herewith an Information Disclosure Statement based on an International Search Report in the corresponding PCT application.

Oath/Declaration

A new Oath/Declaration in compliance with 37 CFR §1.67(a) identifying this application by application number and filing date is submitted, which identifies the effective filing date of the first provisional application, namely, August 7, 1997 and corresponding US applications.

The Examiner comments (Office Action, page 5)

> That the instant claimed subject matter of the elected invention is characterized, as: "namely the use of the intergenic spacer sequences of the chloroplast genome, such as the intergenic spacer 2 region between the tRNA-isoleucine and tRNA-alanine genes, in a universal chloroplast expression vector, was not disclosed in parent application Serial No. 08/591,407 or any earlier parent application. Accordingly, this subject matter is being assigned an effective filing date of the first provisional application, namely August 7, 1997.

Other than "this subject matter", this application benefits of the earlier filing dates identified on this application, e.g., on page 1.

Photographs/Colored Drawings

Applicant notes that the Petition Under 37 CFR §1.84(a)(b) and 1.84(b)(1) filed 15 May 1998 to accept the color and blank and white photographs has been granted. Applicant has amended the "Description of the Drawings" in the specification in accordance with instructions by the Examiner on page 6 of the Office Action.

Terminal Disclaimer

It is noted that a timely filed Terminal Disclaimer in compliance with 37 CFR §1.321(c) may be used to overcome the three double-patenting rejections.

Claims 2-84, 86-96, 107, 118-119, 122, 168-169 and 171-176 are rejected under the judicially created doctrine of obviousness-type double patenting as being unpatentable over claims 19-23, 25-29, 31 and 34 of U.S. Patent No. 5,932,479 and copending applications Serial No. 08/972,901. The undersigned respectfully requests that the Examiner defer the double-patenting responses to the rejections until the other issues in this application are resolved.

Likewise, the Examiner is respectfully requested to defer the double-patenting response to the rejection with respect to claims 31-84, 118-199 and 122 on copending application Serial No. 09/356,192 until the other issues in this application are resolved.

Rejection Under 35 USC §112, second paragraph

Claims 16-30, 45 and 119 are rejected under 35 USC §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states:

> Claims 16-30 and dependent claim 45 are indefinite in the recitation of "other than that of the target plant" which fails to further limit claims drawn to a vector *per se*. Even though the intended use of the vector may be to transform a variety of plant species, the vector itself will still contain DNA sequences from a particular plant species, which may be considered to be from the same target plant or from a different target plant, depending upon the actual use of the vector at any given time. Furthermore, claims 28-30 are indefinite in their recitation of "from a plant other than the target plant from the same species as the target plant species" which is unduly narrative and confusing.

Claims 16-30 and 45 which depend on claim 4 (which is amended) overcome the Examiner's rejection of 35 USC §112, second paragraph. Claim 4, as amended, calls for "....genome of a higher plant, which plant is the same as or different from a target higher plant..." It is submitted that these claims are now in conformance with 35 USC §112, second paragraph.

Claim 119 has been rejected as being "indefinite" in its recitation of "harvested". Harvested means "to gather a crop from (a field or orchard, for example)." "The American Heritage Dictionary of the English Language", page 603. William Morris, Editor. (copy attached). The term is clearly "definite", and well understood.

The difference between claims 118 and 119 is that claim 118 is generic as compared to claim 119. Claim 118 calls for a stably plant which has been transformed with a chloroplast genome stably transformed with a vector of claim 107, which is harvested or not, e.g., a plant which is growing. A plant of claim 119 is a harvested plant.

Enablement - 35 USC §112, first paragraph

Claims 2-84, 86-96, 107, 118-119, 122, 168-169 and 171-176 are rejected under 35 USC §112, first paragraph.

The Examiner states:

> Because the specification, while being enabling for claims limited to an expression vector comprising the tobacco chloroplast intergenic spacer 2 region between the tRNA-isoleucine and tRNA-alanine genes for homologous recombination with the chloroplast genome of higher plants, does not reasonably provide enablement for claims broadly drawn to an expression vector comprising an intergenic spacer from any region of the chloroplast genome from any plant species, or for transformed lower plants such as algae or for the obtention of homoplasmy within a single or multiple generation following chloroplast transformation therewith. The specification does not enable any person skilled in the art to which it pertains, or

with which it most nearly connected, to make and/or use the invention commensurate in scope with these claims.

The specification only provides guidance for an expression vector comprising the tobacco chloroplast intergenic spacer 2 region between the tRNA-isoleucine and tRNA-alanine genes for homologous recombination with the chloroplast genome of higher plants. No guidance is provided for the identification or isolation of any other chloroplast intergenic spacer region from tobacco, any other chloroplast intergenic spacer region from any other plant species, or the transformation of chloroplasts of lower plants such as algae by using said expression vector. Furthermore, no guidance is presented for the actual obtention of homoplasmy, either in single generation following transformation or multiple generations.

The applicant respectfully traverses the rejection. The Examiner observations regarding such "algae" has been noted. All claims as amended are drawn to "higher" plants.

Claims 2, 4 and 86 and newly added claims have been amended as follows:

-Claim 2, line 7 <u>which originate from a plant species the same as or different from the target plant, said sequences being conserved in all higher plants and complementary to the corresponding chloroplast sequences of the target plant, which sequences are also competent of undergoing homologous recombination with said complimentary sequences of the target plant</u>. Claim 4, line 3 <u>a higher plant, which plant is the same as or different from the target plant</u>. Claim 86, line 12 <u>competent of undergoing homologous recombination with complementary spacer 2 sequences of heterologous target plant species</u>.

The prior art has well described in the patent application. Failure of workers which the Examiner cites, including Chasan, has been noted in the instant specification. Pages 4-8. Applicant has overcome the failure of the prior art. The Examiner points out the fact that transformation of monocotyledonous plant has never been reported. Yet see the

specification, pages 43-48, for the transformation of corn, rice and Figures 10A-13A. Prior studies have been failures. See the discussion on the Prior Art Concept of the Intergenic Spacer Regions. Pages 7. The Examiner cites the failures of the "prior art, e.g., Bonnard, Massenet and Laversin. But the invention has overcomed the prior art while making this invention. In view of the state of the art, the invention is new and unobvious!

The patent application, contrary to the dogma for lack of conservation of the spacer regions, uses spacer regions that are highly conserved between different plants to construct vectors competent to transform a variety of higher plants. See page 8, paragraphs 1 and 2; pages 9-11. A method for transformation and to Construct the Universal Vector is well described on pages 11-13. Figures 4A-4G shows the sequence alignment of the spacer (64 bp) region 16S-23S rDNA from several crop species with the tobacco chloroplast sequence where (+) represents the positive and (-) the negative strands, respectively. See Figures 10A-10G, 12A-12F. Note rice and maize (monocotyledonous) transformants. Various plants are illustrated: epifagus, tobacco, helianthus, denothera, alnus, rice, maize, soybean, pea, spinach, cuscuta. Figures 4F-4G. The patent application is rich in guidance for one skilled in the art. See Identification of Intergenic Spacer Sequences. Pages 27-28. Expression of Non-Plant Molecules from Transformed Plants like protein-based polymers (PBPs), like insulin or human serum albumin. Pages 29-33. Variety of plants transformation are exampled. Examples 2-9. Transformed plants which are heteroplasmic and homoplasmic plants are described. Progeny is described (seeds). Pages 59-60. Data is provided, pages 53 (second generation). Analysis and data (and Figures) are detailed: PCR analysis, Northern Blot, Western Blot Analysis, etc. Pages 52-59.

Even the construction of a synthetic universal chloroplast Construction of a Universal Chloroplast Integration Vector Containing a Synthetic Spacer 2 Region is described. Pages 41-42. See claim 193.

This specification does enable any person skilled in the art to which it pertains, or which it is most nearly connected, to make and/or use the invention commensurate in scope with the claims.

Rejections Under 35 USC §102(b)

BIOTECHNICA - Cannon

Claims 2,3, 86-87, 89-94 and 176 are rejected under 35 USC §102(b) as being anticipated by EP 251,654 (BIOTECHNICA - Cannon). Office Action, pages 13-14.

The Examiner states the following:

> BIOTECHNICA (Cannon) "teach the transformation of corn or tobacco with an expression vector comprising a heterologous antibiotic resistance gene under the control of a promoter, flanked by an intergenic spacer region from maize or tobacco for homologous recombination with the chloroplast genome of the target plant (see, e.g., Figures 1a, 1b and 2; columns 3-5, 9, 12, 13 and 15-16; claims 1-18)." The expression vector would inherently comprise a sequence 3' from the structural gene which could be characterized as some type of unspecified "control region." (Underscoring provided)

The rejection on Cannon is respectfully traversed. Section §102(b) the statutory bar provision applies when "the INVENTION was patented described in a printed publication,...more than one year before the first inventor's application filing date.

> It is clearly established that novelty is lacking (i.e., there is anticipation) only when the prior art product or process is identical to that of the inventor's product or process. Such identity exists when the claim of the patent "reads on" the prior art patent or process (i.e., the prior art matter

would infringe the patent claim if it occurred after the patent issued). Chisum on Patents, Vol. 2, 6-29, §602[3]

There is no "identity" with Cannon and the claims rejected. The claims rejected on Cannon call for a "universal integration and vector competent for stably transforming the chloroplast genome of higher plant species....Cannon does not disclose "stably" transforming ....by a "universal" vector. Cannon uses only a spacer for maize or tobacco. There is no "identity" when there is "inherently" and/or "a gene which could" be characterized as some type...", which the Examiner states in the quote above.

The rejection on §102(b) is improper, it is submitted, and a 35 USC §103(a) would also not be proper. (Office Action page 5).

The claims also distinguish over the prior art. Cannon describes a transposon-mediated chloroplast transformation of tobacco for instance, using the bacterial transposon Tn5. Unlike Cannon, no transposon is involved in the transformation of the chloroplast in accordance with the invention. Page 9, lines 17-19. Claims 171 and 194 address this distinction over the prior art. Furthermore, the transposon includes "a gene encoding a selectable maker protein and having insertion sequences on either side of said gene encoding said selectable marker portion, said vector further comprising, on either side of said insertion sequences, a first and second DNA region which together comprise a DNA region homologous with a DNA region of said genome of said plastid." Column 2, lines 35-43. If the transposon were not used in Cannon, the selectable marker protein which is necessary to monitor the transforming, would be absent.

3/14/01 bff

Another essential feature of the Cannon vector is that the vector integrates into a transcriptionally silent region of the chloroplast genome.

Cannon teaches

> Integration of the vector carrying the desired heterologous gene into the genome of the chloroplast is facilitated if the vector either contains DNA heterologous gene into the genome of the chloroplast is facilitated if the vector either contains DNA homologous with a region of chloroplast DNA, or includes a transposon, or both, although integration can occur with neither at a certain, albeit lower, frequency. Integration via homologous DNA occurs via classical homologous crossing over, while integration via transposon DNA does not involve the classical crossing over, and does not require regions of extensive DNA homology. Column 4, line 21-32.
>
> Thus, random chromosomal site integration using transposon DNA will result in the integration of the desired DNA in the chromosome at some sites which are advantageous, and other sites which deleteriously disrupt essential chloroplast genes. Therefore, it is advantageous to direct the integration of the transposon containing the desired DNA to a site in the chloroplast genome where the transcriptional integrity of the crucial native genes will be preserved. To achieve this, the transposon, prior to insertion, is inserted into a fragment of chloroplast DNA (cpDNA) such that the insertion occurs in a region known to be transcriptionally silent. Column 4, lines 38-51. (Underscoring provided).

The vector of Cannon containing the transposon is targeted at a chromosomal region known to be a "transcriptionally silent" region in order to preserve the transcriptional integrity of the native genes. See, the instant specification, page 5, lines 27-33. The insertion of the transposon containing the desired DNA to a site in the chloroplast genome where the transcriptional integrity for the of the crucial native genes will be preserved. Column 4, lines 45-47. Cannon teaches that the transposon is inserted in a suitable such region is one between two known promoters of chloroplast genes, e.g., the promoters for the genes for the chloroplast "large subunit of ribulose bisphosphate carboxylase" and for beta-ATPase. Column 4, lines 51-55. This region is not the region which is called for the

instant claims. Cannon teaches the insertion of the transposon can be directed by either reciprocal recombination or, preferably, homogenization (i.e., gene conversion) of the transposon. Thus insertion can be directed to a locus, preferably a transcriptionally silent region, at which gene inactivation and subsequent loss of function are not caused. Column 5, lines 6-11.

The claims address the distinction of "transcriptionally silent region." The instant specification, page 5, lines 30-35; page 6, lines 3-6; lines 11-15. Claims 190-191; 196-197.

There is another distinction over Cannon. In Cannon no transcription terminator is provided. See the instant specification, page 6, lines 3-5, lines 12-14. Claims 131 and 198 address this feature (middle of the claim).

It is clear that the reference to Cannon does not teach or suggest the subject matter claimed under 35 USC §102(b) or §103(a). It is respectfully requested that Cannon be withdrawn as a reference.

Zoubenko et al.

Claims 2-3, 86-87, 90, 92, 94, 171, 173 and 176 are rejected under 35 USC §102(b) as being anticipated by Zoubenko et al. (Zoubenko). Office Action, page 12.

The Examiner states the following:

> Zoubenko et al. teach an expression vector comprising an heterologous uidA gene or a heterologous aadA gene encoding a polypeptide which confers a selectable resistance to an otherwise lethal antibiotic, said gene under the control of a 5' Prrn promoter and 3' Trps 16 polyadenlyation signal, wherein the vector also comprises a region of homology comprising the tobacco trnV-rps2/7 intergenic spacer region, wherein the genes are conserved throughout the plant kingdom, and wherein the region of homology resulted in stable integration of the heterologous construct into the chloroplast

> genome of transformed tobacco plants (see, e.g., page 3819, Abstract and full paragraph of column 2; page 3829, column 2, top and bottom paragraphs; page 3821, Figures 1 and 2; paragraph bridging pages 3823 and 3824). (underscoring provided)

Applicant respectfully traverses this rejection. The remarks regarding Section §102(b) (see above), are restated. Also a rejection under §103(a) would not be proper. The claims are patentable over Zoubenko.

Zoubenko shows transformation of only tobacco chloroplasts with a vector containing targeting sequences derived only from tobacco. Zoubenko is totally silent regarding a targeting sequence from one species to be used with other plant species. The Examiner states that Zoubenko shows that the tobacco trnV-rps2/7 intergenic spacer region that is being used is "conserved throughout the plant kingdom." It is respectfully noted that there is no such statement in Zoubenko.

Zoubenko emphasizes that the spacer region should have no readthrough transcription region (transcriptionally silent spacer region). Note page 3820, bottom of second column.

> In choosing a targeting region, we first sought a transcriptionally silent location for insertion of foreign genes. Since transcription is divergent from the trnV gene (22) and rps 12/7 operon promoters (23, 24) the trnV-rps12/7 intergenic region was modified for targeting foreign genes. (underscoring provided)

This "transcriptionally silent" is a same region that Cannon teaches.

Further, Zoubenko teaches

> The utility of the pPRV vectors was confirmed by testing: (i) whether the plastid genomes carrying insertions in the trnV-rps7/12 intergenic region are stable and (ii) whether the MCS was inserted in a transcriptionally silent location. Experiments addressing these

issues are described below. (page 3820, column 2, page 3821, column 1) (underscoring provided)

See the passage page 3822, bottom left column showing the test for readthrough transcription into transgenes located in the MCS. Zoubenko teaches that there is no detectable readthrough transcription. "The pPRV target side is therefore uniquely studied to the study of transgene promoter activity using reporter genes. Page 3823, left column. See also the Abstract, the last sentence. This is the equivalent distinction (transcriptionally silent) made over Cannon.

It is respectfully requested that Zoubenko be removed as prior art.

Claims 118 and 122 (drawn to transformed plant) are rejected under 35 USC §102(b) as anticipated by or, in the alternative, under 35 USC §103(a) as obvious over Zoubenko et al. Office Action, pages 14-15.

The Examiner states as follows:

> Zoubenko et al. teach stably transformed tobacco plants comprising transformed chloroplasts as discussed above. The plants taught by the prior art differ from the claimed plants in the method of making them, namely the use of an expression vector comprising a flanking sequence comprising the intergenic spacer 2 region comprising two tRNA genes. However, the use of said expression vector would not confer a unique property to the resultant transformed chloroplasts or plants containing the chloroplasts, since the homologous recombination would not result in the introduction of any new sequences other than the heterologous DNA of interest. Thus, the claimed invention was clearly prima facie obvious, if not anticipated by Zoubenko. See *In re Thorpe*, 227 USPQ 964, 966 (Fed. Cir. 1985), which teaches that a product-by-process claim may be properly rejectable over prior art teaching the same product produced by a different process, if the process of making the product fails to distinguish the two products.

The rejection under 35 USC §102(b) or under §103(a) is traversed. The plants claimed do differ from the prior art not by the method only, but only because the stably transformed plant carries the chloroplast DNA sequences flanking each side of the expression cassette comprising the intergenic spacer 2 region between the tRNA-isoleucine and tRNA-alanine genes which originate from a plant species from the same as or different (donor plant) from the target plant (target). The transformed target plant has a hybrid chloroplast of the donor and the target. Therefore, the plant contains "new sequences (other than the heterologous DNA of interest)". The transformed plant does distinguish over the donor plant. Furthermore, the transformed plant can generate a progeny which contains the same sequences as the transformed donor. See claims 200-201.

Claims 118 and 122 (drawn to transformed plant) are rejected under 35 USC §102(b) as anticipatec by or, in the alternative, under 35 USC §103(a) as obvious over BIOTECHNICA - Cannon. Office Action, page 15.

The Examiner states as follows:

> BIOTECHNICA teaches stably transformed tobacco plants comprising transformed chloroplasts as discussed above. The plants taught by the prior art differ from the claimed plants in the method of making them, namely the use of an expression vector comprising a flanking sequence comprising the intergenic spacer 2 region comprising two tRNA genes. However, the use of said expression vector would not confer a unique property to the resultant transformed chloroplasts or plants containing the chloroplasts, since the homologous recombination would not result in the introduction of any new sequences other than the heterologous DNA of interest. Thus, the claimed invention was clearly prima facie obvious, if not anticipated by, BIOTECHNICA. See *In re Thorpe*, 227 USPQ 964, 966 (Fed. Cir 1985), which teaches that a product-by-process claim may be properly rejectable over prior art teaching the same product produced by a different process, if the process of making the product fails to distinguish the two products.

This rejection is virtually identical as the rejection of these claims over Zoubenko. The response to the rejection for Zoubenko is restated.

The rejection on Cannon is respectfully requested to be vacated for the reasons submitted above with respect to Zoubenko.

Staub et al.

Claims 2-9, 16-21, 31-36, 46-48, 55-57, 61-63, 86-87, 90, 92, 94-96, 107, 118, 122, 168-169, 171, 173-175 are rejected under 35 USC §102(b) as being anticipated by Staub et al (1993) in light of Staub et al (1992). Office Action, page 13.

The rejection as being anticipated on these two references is respectfully traversed.

The Examiner states as follows:

> Staub et al (1993) teach the transformation of tobacco cells with an expression vector comprising a heterologous uidA gene encoding the beta-glucuronidase polypeptide which was subsequently isolated, said gene under the control of a 5'psbA promoter and a 3' psbA polyadenylation signal, wherein the vector also comprises the region of homology found on vector pJS75, wherein whole plants were obtained which demonstrated stable incorporation of the heterologous gene into the chloroplast genome due to homologous recombination with the region of homology (see, e.g., page 601), Abstract and bottom paragraph of column 2; page 602, Figures 1 and 2; page 603, Figure 3B).
>
> Staub et al (1992) show that the region of homology from vector pJS75 in fact contains the tobacco tRNA(Ile) and tRNA(Ala) genes and 16SrDNA gene containing an antibiotic resistance gene, and inherently contains the spacer 2 intergenic region therebetween, and state that the vector has been used for stable introduction of the heterologous uidA gene (see, e.g., page 39, Abstract and bottom paragraph of column 2; page 40, Figure 1 and column 1; page 43, column 2, bottom paragraph).

Staub (1992 in view of 1993) also do not anticipate the claims; nor would a §103(a) rejection apply. In the 1993 paper, Staub discusses recombination between species, tobacco sequences between trnV and 16S RNA, not in the spacer 2 region between trnA and trnI. There is no indication of the utility of the spacer 2 region in this paper, nor of its use to transform plant species other than tobacco.

The 1992 paper also is limited to the transformation of tobacco using a vector containing targeting sequences derived from tobacco. No example is shown of a vector containing an expression cassette with a heterologous sequence. There is no universal capability shown. The restriction site markers created at sites expected not to interfere with the normal functioning of the plasmid genome. Page 39, column 2, paragraph 1.

The Examiner states that the vector of Staub inherently possesses spacer2. But there is no teaching that spacer2 alone can function as a targeting sequence to induce homologous recombination. Indeed, Staub 1992 state in the Abstract that "the integration of long uninterrupted regions of homologous DNA, rather than small fragments..., is the more likely event in plastid transformation of land plants." This would teach the skilled artisan to use long targeting sequences for transformation; actually would lead the skilled artisan away from using a small targeting sequence like spacer2. Therefore, the skilled artisan even further lead away from using spacer2 as a targeting sequence since it may be unstable in the transformed products.

Clearly the two papers by Staub should be withdrawn as a reference, neither as a 35 USC §102; nor would a §103(a) rejection apply. The papers teach away from the claimed invention.

Rejections Under 35 USC §103(a)

Claims 2-84, 86-96, 107, 118-119, 122, 168-169 and 171-176 are rejected under 35 USC §103(a) as being unpatentable over Zoubenko taken with Takaiwa et al., further in view of each of Perl et al and Gordon-Kamm et al, further in view of Maliga et al.

The Examiner states:

> Zoubenko teach the advantages of chloroplast transformation with a vector encoding a peptide of interest and/or selectable marker gene, and comprising an intergenic region for homologous recombination as discussed above.

The Examiner further states:

> Zoubenko does not teach the intergenic spacer 2 region between the tRNA(Ile) and tRNA(Ala) genes, or the transformation of plants and other tobacco.

The Examiner attempts to combine Zoubenko with each one or a combination of these other references. There is no suggestion in any one or a combination of the secondary prior art which teaches to be combined with Zoubenko.

Takaiwa et al. There is no teaching or suggestion to combine the spacer 2 region with the primary reference, Zoubenko. Perl et al. There is no teaching or suggestion which might be combined with the primary reference, Zoubenko. Gordon-Kamm et al.

This article reproduces a system for transgenic maize plants. There is nothing in that article which suggests to be combined with the primary reference, Zoubenko. Maliga et al. There is no teaching or suggestion which might be combined with the primary reference, Zoubenko. Maliga discusses technique manipulating tobacco. There is no teaching which is relevant to the subject claimed. This application discloses and claims method for transforming to which the Maliga reference makes no teaching in this article.

The Examiner in his last sentence on page 17 of the Office Action.

The Examiner states that

> "the choice of additional flanking genes or the inclusion of chloroplast origin of replication."

These secondary prior art references do not suggest any combination with Zounbenko.

The rejection of Zoubenko and one or more of the secondary references as proposed on page 17 of the Office Action is to be removed.

CONCLUSION

For the reasons submitted, applicant respectfully request a favorable review of this application and an allowance.

Respectfully submitted,

Gerard J. Weiser
Reg. No. 19,763
For SCHNADER HARRISON SEGAL & LEWIS LLP
(215) 751-2427

Date 10/26/00